The Bacteriology of Flax Retting: GEORGE T. MOORE, Ph.D., of West Chester, Pa.

Note as to the Measurement of the Action of Water upon Zinc and Lead: DR. WILLIAM PITT MASON, of Troy, N. Y.

New Results in Electro-Analysis: Dr. EDGAR F. SMITH, of Philadelphia.

The Production of Synthetic Alcohol: Dr. HARVEY W. WILEY and HERMAN SCHREIBER, of Washington, D. C.

The Progress of the Isthmian Canal: Dr. ELIHU THOMSON, of Swampscott, Mass.

On the Transportation Crisis: Professor LEWIS M. HAUPT, of Philadelphia.

The Princeton Archeological Expedition to Syria: HOWARD CROSBY BUTLER, of Princeton. (Lantern illustrations.)

The Association Theory of Solutions: Professor WILLIAM F. MAGIE, of Princeton.

The Groups which are Generated by Two Operators of Order Two and Four respectively, where Commutator is of Order Two: Professor G. A. MILLER, of Urbana, Ill.

SATURDAY, APRIL 20

Santa Cruz Typotheria: W. J. SINCLAIR, of Princeton. (Lantern illustrations.)

Santa Cruz Birds: MARCUS S. FARR, of Princeton.

Restorations of Santa Cruz Mammals: Professor WILLIAM B. SCOTT, of Princeton.

On the Temperature, Secular Cooling and Contraction of the Earth, and on the Theory of Earthquakes held by the Ancients: Dr. T. J. J. SEE, of the U. S. Naval Observatory, Mare Island, California.

On Continental Development: BAILEY WILLIS, M.E., of Washington.

A Study of the Mean Temperatures of the Surface of the Moon, Earth and other Planets: Professor CLEVELAND ABBE, of Washington.

The Solar Corona: W. W. CAMPBELL, of Lick Observatory, California. (Illustrated.)

Astronomical Photography: Professor E. E. BARNARD, of Yerkes Observatory, Williams Bay, Wis.

Conservative Systems with Prescribed Trajections: Professor E. O. LOVETT, of Princeton.

Comparison of Results of Observations at the Flower Observatory for the Years 1905 and 1906, with the Wharton Reflex Zenith Tube and the Zenith Telescope: Professor C. L. DOOLITTLE, of Upper Darby, Pa.

Comparison of Results of Latitude Observations at the Sayre Observatory, South Bethlehem, and at the Flower Observatory, Philadelphia, from September 30, 1904, to September 3, 1906: J. H. OGBURN, of Bethlehem, Pa.

Two Remarkable Double Stars: (a) the short period Binary, Hough 212, and (b) the Stellar System Krueger 60: ERIC DOOLITTLE, of Upper Darby, Pa.

THE SOCIETY OF AMERICAN BACTERIOLOGISTS

THE eighth annual meeting of the Society of American Bacteriologists was held in New York during Convocation Week. The meetings were held at the College of Physicians and Surgeons, and at the Rockefeller Institute, the latter in conjunction with Section K of the American Association for the Advancement of Science.

The first session of the society was held on Thursday morning, December 27, in the main lecture room of the College of Phy-

sicians and Surgeons, and, after a short business meeting for the reading of reports and the nomination of new committees, a number of interesting papers were presented on various general aspects of bacteriology, morphology, physiology, etc. The joint meeting at the Rockefeller Institute on Friday morning was devoted largely to the discussion of subjects of interest alike to the bacteriologist and the pathologist and chemist. The papers at this session were largely devoted to various investigations of sera, toxins, and the biology of pathogenic bacteria and other organisms. The third session, on Saturday morning, at the College of Physicians and Surgeons, dealt with laboratory procedure, methods of cultivation of certain bacteria, and studies on the bacteriology of milk.

The following officers were elected for the present year:

President—Dr. James Carroll.
Vice-president—Professor F. D. Chester.
Secretary-Treasurer—Professor S. C. Prescott.
Council—Dr. J. J. Kinyoun, Dr. F. G. Novy, Dr. F. P. Gorham, Dr. H. W. Conn.
Delegate to the American Association for the Advancement of Science—Dr. Erwin F. Smith.

The papers, of which abstracts, or titles alone, are presented below, were as follows:

Movements of Certain Bacteria in Soils: Karl F. Kellerman and Edna H. Fawcett, Bureau of Plant Industry, Department of Agriculture, Washington, D. C.

Two organisms have been studied in connection with *Pseudomonas radicicola, Bacillus ochraceus* and a form resembling *Bacillus coli.* The latter kills *Pseudomonas radicicola* when in synthetic nitrogen-poor sugar bouillon, but not in soil extracts of favorable soils; *Bacillus ochraceus* has little effect upon *Pseudomonas radicicola* under either condition.

In sterilized favorable soils saturated with water *Bacillus ochraceus, Pseudomonas radicicola,* and the paracolon organism grow with almost equal speed, progressing about one inch in forty-eight hours. In soils barely moist *Pseudomonas radicicola* progresses at the rate of about one inch in seventy-two hours, while the two other forms are reduced to a rate of about one inch in eight days. These experiments were all conducted at a temperature of 25° C. At a temperature of 10° C. the rate of *Pseudomonas radicicola* was reduced to one inch in three days in saturated soil; the two other organisms had made practically no growth at the end of thirty days.

Under none of the conditions of these experiments did there seem to be any antagonism in the soil between *Pseudomonas radicicola* and *Bacillus ochraceus* or the paracolon organism.

Further Studies on Putrefaction: Leo F. Rettger, Yale University.

1. Real putrefaction is caused only by obligate anaerobes. None of the obligate aerobes and facultative anaerobes thus far studied have revealed this property. Some organisms like *B. pyocyaneus* and *Proteus vulgaris* are able to slowly digest coagulated egg albumin and blood serum, but the products are not those of putrefaction. Mercaptan in particular is invariably absent.

2. *B. putrificus* (Bienstock), the bacillus of malignant œdema and of symptomatic anthrax are putrefactive organisms, though they may vary at times in this respect. None of the strains of *B. tetanus* so far examined have shown any putrefactive action in the so-called egg-meat medium.

3. *Bacillus aerogenes capsulatus* of Welch (or *B. enteritidis sporogenes* of Klein) is essentially a fermentative organism. At no time has it been able to cause any apparent decomposition of coagulated proteids, although cultures of the organism often have a decided putrefactive odor.

4. Normal feces in a large percentage of cases caused a marked decomposition of the egg-meat medium. In a large number of such cases the *Bacillus putrificus* of Bienstock was observed to be responsible. In at least 25 per cent. of the stools examined the bacillus of malignant œdema was present, though in small numbers. The amount of feces tested at one time varied from 2 to 32 milligrams. The *Bacillus aerogenes capsulatus* of Welch was found to be present constantly when the quantity of feces examined was at least 32 milligrams. In 2 milligram quantities, however, it failed to reveal itself in at least 50 per cent. of the tests that were made with such small amounts.

A Study of the Variation in the Biochemical Reactions produced by Cultures of the Colon Type: STEPHEN DEM. GAGE.

The study was made to ascertain how closely the variations in the intensity of certain biochemical functions of cultures usually included in the colon group by routine tests would agree with the law of biological variation.

About 200 cultures of the colon type, as interpreted at the Lawrence Experiment Station, were examined after incubation at 40° C. for the amount of gas produced in dextrose broth in 24 and 48 hours, for the proportion of CO_2 in that gas, and for the amount of ammonia and of nitrites produced in nitrated pepton solution in 24 hours.

The amount of gas produced in 24 hours varied between 15 per cent. and 100 per cent., about four fifths of the cultures producing between 40 and 70 per cent. of gas.

After 48 hours' incubation the amount of gas also varied between 15 per cent. and 100 per cent., and the distribution of the cultures among the different gas values was more uniform than at the end of 24 hours, about half of the cultures producing between 35 per cent. and 50 per cent. of gas. With 15 per cent. of the cultures the amount of gas increased between the first and second day, while with 65 per cent. of the cultures there was a decrease in the amount of gas on the second day.

The amount of CO_2 in the gas at the end of 48 hours varied from none to 75 per cent., about three fourths of the tubes containing between 10 and 25 per cent. of CO_2.

In the nitrated pepton solution at the end of 24 hours about 30 per cent. of the tubes contained less than one part per million nitrogen as ammonia, and about half of the tubes contained between one and three parts, while about one fourth of the tubes contained less than one part nitrogen as nitrites, and about half of the tubes contained between one and three parts.

The number of determinations was altogether too small to plot curves of biological variation with any degree of accuracy. The curves plotted, however, appear to be simple, unimodal curves, and indicate that the group was a true biological group and that the variations were normal biological variations.

Involution and Degeneration Forms of Bacteria: D. H. BERGEY, M.D.

The indefinite and confusing definitions of the nature of involution and degeneration forms of bacteria found in most textbooks on bacteriology are bewildering to the student, and leave him in doubt as to the exact significance of these terms.

The term 'involution forms' is defined in the Standard Dictionary as 'certain swollen, bladder-like and irregular forms which the organisms sometimes assume after their death, or as the result of deleterious influences, such as insufficient nutrition.' This definition is sufficiently clear,

and is in general use by authorities in textbooks on bacteriology.

The term 'degeneration forms' is, however, less definitely defined in the textbooks. The Standard Dictionary defines degenerate, ' to become worse or inferior; decline in character, qualities, or excellence, as from the normal or primitive condition or from a type of standard; deteriorate.' According to this definition, the term 'degenerate forms' should be reserved for those organisms that had reached the normal stature and subsequently manifested the effects of degenerative influences. These effects are seen principally as vacuolation, granulation, and fragmentation of the protoplasm in organisms of normal size and shape.

The Production of Indol in Proteid-free Media: M. X. Sullivan, Brown University, Providence, R. I.

Several years ago, I found that *B. coli communis* would grow well on non-proteid media. At that time no tests were made to determine whether or not indol and skatol were produced on these media. Under the pressure of other work the experiments on *B. coli* were not continued. Recently, however, my attention was again directed to the metabolism of *B. coli* by L. F. Rettger's article ' Studies on Putrefaction,' *Jour. of Biol. Chem.*, '06, Vol. II., p. 71. Accordingly solutions of non-proteid media were made and inoculated with a pure culture of an indol-forming variety of *B. coli* in order to determine whether or not this bacillus could produce indol in simple media.

The culture medium on which the present conclusions are based consisted of

Asparagin	0.2	grm.
Mannite	0.2	
NaCl	0.02	
$MgSO_4$	0.01	
$CaCl_2$	0.02	
K_2HPO_4	0.2	
H_2O	100	c.c.

This medium was made slightly alkaline to litmus and divided into two portions of 2,000 c.c. each: flask (*A*) and flask (*B*) respectively. To (*B*) was added $CaCO_3$ to take up whatever free acid might be formed. Both portions were then sterilized and inoculated with *B. coli.*

In periods varying from five to ten days the contents of (*A*) and (*B*) were analyzed for indol and skatol. In no case was indol or skatol found, either in the distillate or in the original solution. An inoculation from the non-proteid medium containing *B. coli* to bouillon produced in a few days a good growth with the formation of indol.

From these experiments it would seem that indol is not formed synthetically, but in ordinary culture media is a result of the decomposition of the albuminous material present. Long-continued growth of *B. coli* on a non-proteid medium I believe will give a test for indol, since the death of many micro-organisms will leave an albuminous material in the medium.

The Sterilization of Sewage-Filter Effluents: Earle B. Phelps.

The development of the modern, rapid processes for the purification of sewage has led to a new conception of the functions of a sewage-disposal plant, in which conception the removal of bacteria finds but small consideration. The cost of obtaining high bacterial efficiency, by means of sand filtration, is so much greater than the cost of producing an effluent which is non-putrescible, though germ-laden, that the former can not as a rule be employed in the treatment of the sewage of large cities, unless bacterial purification is imperative. In certain cases, as for example where important shell-fish industries are threatened, it does become necessary to consider bacterial as well as chemical purification. As an alternative to treatment on sand, either as the chief, or as a supplementary process,

chemical sterilization of the rapid filter effluents has been proposed. Experiments carried out by the writer at the Sewage Experiment Station of the Massachusetts Institute of Technology seem to indicate that the process is much more feasible than has hitherto been supposed, and that it can, in fact, compete with supplementary sand filtration, as a finishing process for the removal of bacteria from effluents. The most suitable disinfectant thus far tried is chloride of lime or bleaching powder.

During the past summer and fall the effluent of a trickling filter, treating Boston sewage at a rate of two million gallons per acre per day, was treated with bleaching powder at a rate of five parts of available chlorine per million of effluent. The average removal of total bacteria was 99.96 per cent. and of organisms fermenting lactose in bile media 99.993 per cent. The cost of treatment for chemicals alone is $1.08 per million. The time of contact was two hours. Some special experiments in bottles upon the germicidal action of bleaching powder on *B. typhosus* showed a practical elimination of that organism in from two to four hours, the same amount of available chlorine being taken.

Results with copper sulphate were not so satisfactory. During October the application of one part of copper per million gave a reduction of 94 per cent. of the total organisms and 98.5 per cent. of the fermenting forms. The mean temperature was 56°.

In November, with a mean temperature of 46°, the removal was 79.5 per cent. and 98.4 per cent., respectively.

It was necessary to double the strength of copper, two parts per million, to restore its germicidal value to the first figure, when, in December, the mean temperature was 43°. Under these conditions the efficiency was, for the total organisms, 97.9 per cent. and for the fermenting forms, 98.8 per cent.

The cost of two parts per million of copper as sulphate is about the same as of five parts per million of chlorine as bleaching powder. The germicidal effect of the former is not nearly so great.

Some experiments, previously reported by the writer, showed that the presence of organic matter (sugar or peptone), seriously interfered with the toxic action of copper sulfate on the typhoid bacillus.

A Substitute for Potato as a Culture Medium: P. G. HEINEMANN, University of Chicago.

Ten grams of agar are dissolved in 600 c.c. water. A solution of the following salts dissolved in 200 c.c. water is then prepared:

Dipotassium hydrogen phosphate	2 gr.
Disodium hydrogen phosphate	2
Magnesium sulphate	2
Calcium chloride	2
Ammonium lactate	2
Asparagin	5

This solution, in which a fine precipitate forms, is added to the hot agar solution, 10 grams peptone dissolved, and the whole mixture filtered after the reaction, which is about 5 per cent. acid, is brought to the neutral point with phenol phthalein.

To the hot filtered solution 30 grams of starch, previously washed in water and made perfectly homogeneous in a mortar, is gradually added with constant stirring. The mixture is then brought to near the boiling point and finally weighed. The total weight should be 1,000 grams. The medium is tubed and sterilized in the autoclav for 5 minutes at 120°, and cooled in a slanting position.

The Enzymotic Properties of Diplococcus Intracellularis: SIMON FLEXNER, from the Rockefeller Institute for Medical Research.

The brief vitality of many of the cultures of *Diplococcus intracellularis* is a point of differential importance. Many strains, grown in a favorable medium, unless transplanted to a fresh medium do not survive beyond two or three days. Cultures three days old show marked degenerations, and the latter increase rapidly with age until, at the end of five or six days, or even earlier, no normal cocci persist. As degeneration progresses, loss of staining power and disintegration ensue, until, finally, staining is lost and a formless detritus remains.

The changes in the diplococcus are associated with the action of an enzyme which brings about the disintegration. This enzyme does not exhibit the usual properties of a proteolytic ferment; it does not liquefy gelatin or coagulated serum. The degree and rapidity of its action varies with its concentration; at least a heavy suspension of the cocci in salt solution, kept at 37° C., undergoes dissolution more rapidly and completely than a weaker suspension. The vitality of the cultures is associated with the degree of autolytic alterations in the suspensions; cocci in the weak suspensions survive longer than in the stronger ones. At lower temperatures—2° C.—disintegration of the cocci either does not take place at all or progresses much more slowly. Under the latter conditions more cocci survive in the strong than in the weak concentrations, although even here the vitality is a brief one.

Potassium cyanide restrains the action of the ferment which tends to disintegrate the diplococci; after removal of the cyanide dissolution sets in. Heating the diplococci to 65° C. prevents or reduces the dissolving power of the intracellular enzyme.

The enzyme acts upon the dead cocci—probably not upon the living germs. Diplococci killed by heat (50° to 55° C.) undergo autolysis; but when the cocci are killed by the addition of toluol, autolysis is accelerated. A heavy suspension of the diplococci in salt solution, under toluol kept at 37° C., may be disintegrated in four hours.

The enzyme of the diplococcus acts energetically upon other bacteria, bringing about their dissolution. It acts upon *B. typhosis, B. coli communis, B. pyocyaneus, B. anthracis, M. catarrhalis,* and to a less degree and more slowly upon *Staphylococcus aureus.*

The Stability of Tetanus Toxin: M. J. Rosenau, Hygienic Laboratory, Washington, D. C.

Some Observations on the Blood of Horses: J. J. Kinyoum, Philadelphia, Pa.

The volume percentage of the cell content of normal horse blood ranges from 30 to 46 per cent.; an average of 37.8 per cent. The administration of diphtheria toxin, causes a diminution of the red cells to an average of 30 per cent.

The anæmias induced by the toxin and the subsequent bleedings are progressive, and are in direct relation to the length of treatment. Horses treated with tetanus toxin, or with dead or living micro-organisms, also show a progressive diminution of the corpuscular content, but it is not so great as in the cases when diphtheria toxin is administered.

The danger point for all horses, beyond which the horse can not be bled, is when the cell content falls below 20 per cent. The leucocytes bear no fixed relation to the red cells; it may be as great as 1 per cent. or as small as 0.1 per cent. normal, and the same in those undergoing treatment.

There is also no relation between the cell content and the anti-bodies.

The hæmoglobin follows very closely the curve of the cells (red), both in the normal and in the treated horses.

Toxic Effects of Horse Serum.—Guinea-

pigs sensitized with horse serum begin to respond to the toxic effects of the same serum on the eighth day.

If, however, the sensitized pigs be injected with the serum of a horse which has been injected several times with progressive quantities of human blood, there is no effect.

On the contrary, if the sensitized pig be injected with the serum of a horse which has received repeated injections of horse blood rendered hæmolytic for human blood (red cells), the toxicity is not only restored, but is somewhat more toxic than the normal serum.

The toxic effect of horse serum is modified by the amount of the sensitizer; those which have received as much as 10 to 15 c.c. of serum as a sensitizer, are rendered quite resistant to the toxic dose. When the amount of the sensitizing dose is as much as one tenth the body weight, the pigs are immune.

The toxic effect of the serum is also influenced by the character of the sensitizer; for an example: If guinea pigs are sensitized by a toxin-antitoxin mixture, they are more susceptible than when given normal or antitoxic serum.

The toxic effect of a serum bears a relation to the amount given, and is influenced by the body weight.

Precipitated and dialyzed antitoxic serum is less toxic than the normal, or antitoxic serum.

Milk was found to be nontoxic to guinea-pigs sensitized with antitoxic serum.

The Alleged Rôle of Intestinal Worms as Inoculating Agents in Typhoid Fever: CH. WARDELL STILES,[1] Ph.D., D.Sc., Chief of Division of Zoology, Hygienic Laboratory, U. S. Public Health and Marine Hospital Service.

According to a theory recently advanced in France, intestinal worms (especially whipworms) form the inoculating agent in typhoid, much in the same way that fleas inoculate bubonic plague. The theory is based upon the high percentage of whipworms reported for the typhoid cases by some authors and upon the fact that intestinal worms may wound the mucosa; it is assumed that the uninjured mucosa forms an impassable barrier to the bacteria, which, however, may pass through these wounds. The theory claims that typhoid bacilli in the intestines are harmless unless parasitic worms, or some other wounding agents, are present. Accordingly, the treatment and prevention of typhoid reduces itself essentially to treatment and prevention of parasitic worms, especially of whip-worms. The theory is extended to appendicitis, cholera and certain other intestinal diseases.

More recently, the theory is also extended to include parasitic protozoa as inoculating agents in intestinal diseases, but so far as typhoid is concerned no definite statistical data are presented in support of this extension. As the fresh, warm stools should be examined to test this phase of the subject fairly, and as conditions were not favorable for such examination in the present instance, this protozoan phase of the subject could not be consistently studied in the present report.

The Washington epidemic of typhoid in the summer of 1906 presented the possibility of putting to a practical test the verminous side of this exceedingly alluring theory. The results of the study have failed to confirm the theory, for 92.5 per cent. of the patients showed no infection

[1] In making the 2,000 microscopic examinations involved in preparing this paper I have been aided by Past Assistant Surgeon Joseph Goldberger, David G. Willets, Ph.B., and Arthur E. Paterson, Ph.B.

with intestinal worms, while only 15 of them (7.5 per cent.) showed a total of 16 infections (8 infections per hundred), of which 14 cases (7 per cent.) showed whipworm infection. This represents an increase of only 1.3 infections (0.65 infections per hundred persons) over what we expected to find in the general intestinal helminthiasis, and an increase of only 1.32 per cent. over what we expected to find in whipworm infections. Considering the very wet season we have had, and especially in view of the negative findings in 92.5 per cent. of the patients, these slight increases can hardly be considered of importance.

In comparing the severity of the verminous infections (as judged by the number of eggs present) with that reported for typhoid by Guiart, in France, it was found that the Washington cases averaged only 0.47 + whipworm egg per slide, against two eggs per slide in the French statistics.

Turning to a method of indirect comparison, it is seen that while former examinations in this laboratory (for Washington, D. C., and for Connecticut) showed that the greatest percentage (13.01) of cases of whipworm infections was under fifteen years of age, in the 200 typhoid cases examined the greatest percentage (47.5) of patients fell between the ages of fifteen and thirty years, inclusive; further, the percentage of cases of typhoid does not vary parallel with the percentage of cases of whipworm infection in the other age groups.

Comparing, in reference to sexes, the statistics of whipworm infection in the world at large, and in examinations made for Connecticut and for the District of Columbia combined, with those of the 200 typhoid patients examined, it is seen that whipworms are more common in females than in males, while of our 200 typhoid cases 52.5 per cent. were males and 47.5 per cent. were females. If the comparison is restricted to the total helminthiasis of cases examined in the District of Columbia, it is slightly more favorable to the theory under discussion.

Making a similar comparison in reference to the race of patients, it is seen that in the 200 cases of typhoid under discussion (reduced to figures approximately in harmony with the general relation of the races in the population of the District) the whites were to the negroes as 55.5 to 64, while in the whipworm statistics in former examinations the whites were to the negroes as 3.75 to 9.79. The change in our summer population would account for at least a part of this excess of typhoid among the negroes.

The general conclusions are, therefore, that a study of the intestinal helminthiasis in 200 of the cases in the Washington typhoid epidemic of 1906 has not supported the theory that whipworms, eelworms, or other species of intestinal worm bear any necessary or common relation as an inoculating agent in typhoid fever; and that the view recently expressed in France to the effect that the treatment and prevention of typhoid fever practically reduces itself to the treatment and prevention of intestinal worms, especially of whipworms, does not obtain, at least so far as this locality (Washington, D. C.) is concerned. The question of the relation of protozoa as inoculating agents in typhoid is not considered in this report.

On the Absorption of the Third Serum Component: W. H. Manwaring, Indiana University.

Besides amboceptor and complement, hemolytic serum contains a third active component. This third serum component may possess hemolysis-increasing (auxilytic) or hemolysis-decreasing (antilytic)

properties, depending largely on experimental conditions.

The third component is changed in its hemolytic properties by exposure to washed blood corpuscles. Part, at least, of this change is due to the giving off into the third component of antilytic corpuscle products. No actual absorption of the third serum component by washed corpuscles has as yet been demonstrated.

On the So-called Physical Chemistry of Hemolytic Serum: W. H. MANWARING, Indiana University.

Two physico-chemical laws have been proposed for hemolytic serum: (i.) a law governing the interaction of amboceptor and complement.

When heated hemolytic serum is exposed to washed corpuscles, three phenomena take place: (i.) A hypothetical absorption of amboceptor; (ii.) A change in the third serum component; and (iii.) a giving off into the serum of antilytic corpuscle products. No measurement of the amboceptor-power of exposed heated hemolytic serum will, therefore, give any idea whatever of the amount of unabsorbed amboceptor it contains.

Similarly, the existence of an active third component, which differs in different sera prepared under identical conditions, prevents the experimental proof or disproof of the physico-chemical law proposed for the interaction of complement and amboceptor.

The physical chemistry of hemolytic serum is, therefore, beyond the present power of experimental science.

On the Chemical Inactivation and Regeneration of Complement: HIDEYO NOGUCHI, Rockefeller Institute.

On the Electric Charge carried by Toxins, Antitoxins, and Agglutinins: C. W. FIELD, Research Laboratory, New York Board of Health.

I. *An Improved Technic for Tuberculo-Opsonic Preparations.* II. *Some Suggestions concerning the Terminology of Opsonic Theory and Practise:* A. P. OHLMACHER, Detroit.

Generic Characters in the Coccaceæ: C.-E. A. WINSLOW and ANNE F. ROGERS.

The difficulties in the classification of bacteria arise partly from the variations in certain characters produced by slight changes in the environment, but chiefly from the fact that characters which appear to be constant for the individual exist in an almost infinite number of minute gradations in different races. Extreme differences in any property are completely connected by a series of intermediate forms. The best basis for a natural classification is the statistical study of a large series of individuals, which will disclose the points about which the largest number of races are grouped, which are presumably the type centers round which the organisms vary. A study of 500 cultures of cocci isolated from various sources has been made with this end in view. It has shown that the variations which exist group themselves on normal 'curves of frequency.' These show in some cases a single mode, as in the fermentation of dextrose and lactose, all the cultures studied grouping themselves about a single center, and in other cases more, as in gelatin liquefaction, where two centers exist, one for the liquefying and one for the nonliquefying forms. Chromogenesis shows four centers of variation, the white, yellow, orange and red forms being quite definitely separated.

The most significant result of this study is the fact that differences in the various characters are strikingly correlated with each other and with the source from which the organisms were derived. For example, the orange chromogens are parasitic forms which never show the sarcina grouping, are

generally Gram positive, and liquefy and ferment sugars very actively, while the red chromogens are their opposite in each respect. It is thus possible to divide the cocci into groups, each marked by the prevailing combination of certain characteristics. These groups are defined in relation to the central type about which they cluster rather than separated by sharp boundary lines; yet they certainly mark natural groups and seem to deserve generic rank.

The generic groups thus established are six in number. Streptococcus is characterized by parasitic origins (from the surfaces of the plant or animal body), feeble growth on media, occurrence of cells in chains or small groups, variable response to Gram stain (though generally a positive one), variable acid production in sugars (often reaching very high values), the absence of nitrate reduction, and the general absence of gelatin liquefaction. (The species in this genus have recently been very satisfactorily worked out, by the statistical method, by Andrewes and Horder, *Lancet,* September 15, 1906.) *Aurococcus* (n. gen.) is characterized by parasitic origin, good growth on media, orange pigment, occurrence of cells in irregular groups, generally positive reaction to Gram stain, and the formation of considerable acid in sugar solutions. Nitrates may or may not be reduced, and gelatin is either not liquefied or liquefied strongly. *Albococcus* (n. gen.) differs from *Aurococcus* in producing a somewhat heavier growth of white color, in forming somewhat more acid in sugar solution, and in a less vigorous action on gelatin. These three genera, with *Diplococcus,* which stands at the extreme end of the series beyond *Streptococcus,* may be grouped in a subfamily, the Paracoccaceæ, which includes parasitic forms producing faint to good growths, made up of chains or groups of cells, generally staining by Gram and producing considerable acid.

The rest of the cocci may be grouped together as Metacoccaceæ, including saprophytic forms, producing vigorous surface growths made up of groups of cells or packets, generally Gram negative and showing a slight action on sugars. Here belong the two generally accepted genera, micrococcus and sarcina, the first showing only irregular groups; the second, packets. In both the organisms are most abundant in air, water and earth, rather than on the body; the surface growths on media are abundant and the pigment is yellow; the Gram stain is usually negative and sugars are fermented very slightly. Gelatin is not liquefied, or liquefied somewhat slowly, and nitrates may or may not be reduced. Finally, *Rhodococcus* (n. gen.) includes the red chromogens, which show either groups or packets, rarely liquefy gelatin and reduce nitrates, if at all, only to nitrites and not to ammonia. In other respects they resemble *Micrococcus.* The group of the cocci, as a whole, shows in each character studied a gradual but continuous series of modifications from the strictly parasitic diplococcus to the strictly saprophytic rhodococcus.

The methods used in this study have been described in the *Journal of Infectious Diseases* for June, 1906, and the final conclusions will shortly be presented in the *Journal of Medical Research.*

Actinomyces of the Oral Cavity: D. H. Bergey, M.D.

The occurrence of actinomyces organisms in the oral cavity is believed to be a subject of interest, not only from the standpoint of infection starting from this place, but also on account of the possibility that these organisms may be directly injurious to the teeth.

Four organisms of actinomyces were iso-

lated from healthy mouths, and studied in more or less detail. These organisms resolve themselves in two types: the one a rather short, club-shaped, striated, branching form, and the other a long, filamentous branching form.

These organisms adhere very tenaciously to the culture media on which they are growing, and this property no doubt manifests itself in the oral cavity, where the organisms probably adhere with equal tenacity to the teeth and assist in the formation of plaques. They also have the property of breaking up a number of the carbohydrates, and in this way also exert an injurious effect through the formation of acids, thus contributing to caries.

The study of the pathogenic properties of these organisms is not yet completed.

The Growth and Toxin Production by B. Diphtheriæ upon Proteid-free Media: PHILIP B. HADLEY.

This is the preliminary report of a study whose object was to learn what constituents of proteid-free media were either favorable or necessary for the formation of toxin by *B. diphtheriæ* upon such media. The results thus far attained may be summed up as follows:

1. Very few cultures of *B. diphtheriæ*, fresh from the throats of man, will take up a growth directly upon proteid-free media.

2. Most cultures which will not at first grow upon proteid-free media may be adapted to grow upon such media. The adaptation may be accomplished by gradually diminishing the amounts of bouillon and increasing the amount of proteid-free media in a combined medium consisting of both proteid-free and bouillon until the proteid-free medium itself is reached. Each tube is inoculated from the preceding tube after an incubation of 3–5 days.

3. Whether the growth of *B. diphtheriæ* upon proteid-free media be spontaneous or the result of adaptation, there may be formed a toxin as virulent as that obtained from bouillon cultures.

4. Of the three nitrogen bases tried (asparagin, urea and glycocoll), urea seemed to be of little value, while glycocoll furnished the best growth and strongest toxin. Asparagin appeared to give better results than urea, though it was not as satisfactory as glycocoll.

5. A single morphological variety of *B. diphtheriæ* may be decidedly modifiable; and there are facts which point to the view that there may be, in the life of the diphtherial organism, what we may roughly call a cycle of adaptive forms, each one of which is best suited to a circumscribed condition of environment in which it may or may not produce a virulent toxin.

6. In all cases of growth upon proteid-free media, whether spontaneous or resulting from adaptation, it is the solid-staining varieties of the diphtherial organism which manifest the most luxuriant growth.

On the Cultivation of Spirillum Obermeieri: F. G. NOVY and R. R. KNAPP, the Hygienic Laboratory, University of Michigan, Ann Arbor, Mich.

The *Spirillum Obermeieri* has been maintained in this laboratory, since November, 1905, by a consecutive passage through rats. Although during this time many hundreds of attempts to secure cultures on artificial media have been made they have given uniformly negative results. In the defibrinated blood of infected rats, the spirilla retain their vitality for a variable length of time depending upon the stage of the disease during which the blood is drawn. If drawn during the decline stage, that is to say at a time when the organisms have reached their maximum and are beginning to decrease in numbers, the spirilla will often die out in less than 24 hours. This is due, as we have shown, to the presence of

specific germicidal bodies. On the other hand, in 'onset blood' drawn during the early stage of the disease the spirilla may live for several weeks. Thus, we have seen living spirilla in such blood kept for 30 to 37 days and have been able to infect rats with blood kept for 40 days. Moreover, we have been able to make use of this fact in shipping the virus to distant points, to Dr. Todd at Liverpool and to Professor C. Fraenkel at Halle.

In our first series of attempts at cultivating the spirilla on blood agar we were, as a rule, unable to keep the organisms alive for more than two or three days. Since then, however, we have been somewhat more successful and have kept them on blood agar for 22 to 28 days, and in some experiments now in progress they are still alive and numerous on the thirtieth day. As yet, however, no evidence has been obtained of actual multiplication *in vitro*. The organisms which are found to persist we prefer to regard as mere survivals until actual subcultures have been obtained.

The successful results obtained by Lavaditi in the cultivation of *S. gallinarum*, *S. Duttoni*, and *S. refringens* in collodium sacs led us to apply this method to our spirillum. With this object in view the collodium sacs were filled with rat or rabbit blood, or corresponding sera, heated and unheated, and after inoculation with spirillar blood these sacs were placed in the peritoneal cavity of rabbits. After three to seven days the sacs were removed and contents were examined with *uniformly* negative results. Apparently the rabbit is unsuited for sac cultures.

We were finally led to make the trials under conditions approachng the natural state as much as possible. For this purpose, the collodium sacs were filled with uncoagulated rat blood and after inoculation were placed at once in the peritoneal cavity of a white rat. Three days later, on removal, the sacs were found to contain active spirilla and in increased numbers. From the sacs, transplants were made to new ones and the result was equally satisfactory. The spirilla were found to be in an extremely active condition and were undoubtedly multiplying.

From this time on the transplantations were made regularly, every three or four days, from sac to sac. After a few passages the uncoagulated blood was replaced by defibrinated rat blood or by rat serum. Defibrinated rabbit blood has also been employed to some extent, but whether it will continue to be a favorable medium we are unable to state. Two sacs were inoculated each time and placed in the peritoneal cavity of a rat. Each sac had a capacity of from 2.5–3.0 c.c. and was sealed so as to leave within as little air as possible. It is a noteworthy fact that on removal from the rat the sacs are invariably greatly distended as a result of osmotic changes. Furthermore, the air which was originally present is in large part, and at times wholly absorbed.

Since October 13, the spirilla have been carried through 20 consecutive passages in 68 days, and presumably they can be kept multiplying under these conditions indefinitely. The spirilla in the sac culture are never as numerous as in the blood of rats. They rarely exceed more than 5 to 10 per field of the one twelfth inch objective, as contrasted with several hundred per field met with in the blood of rats during the maximum period of infection. The inoculation of the sac contents (blood or serum) into rats, it is interesting to note, is followed by a mild infection in which the spirilla are not much more numerous than in the sacs. Moreover, in such infection

they persist for a day or two longer than is the case with the active virus.

When the sac is allowed to remain in the rat for seven days the spirilla decrease greatly in numbers and may even disappear. In the culture sacs after removal from the rat, and kept at room temperature, the spirilla die out in a day or two.

Throughout this series the spirilla have preserved their form unchanged. They appear either as single cells (8 microns) or of double length (16 microns) but at times even longer spirals are found. The latter are the result of end to end union by means of flagella as we have heretofore shown. As in the case of blood preparations no evidence is observed of division other than transverse. One observation in this connection is deserving of special emphasis owing to its bearing upon the question as to whether spirochetes multiply by transverse or longitudinal division. In these cultures it is not unusual to find short spirals of two or three turns, and from 4 to 8 microns in length. These may occur singly or in pairs (8 to 12 microns long) showing the pale division zone. The width of the short form is the same as that of the longer cells. The occurrence of these short spirals is readily explainable as the result of transverse division. It may further be stated that the cultural spirals usually stain solid by the Romanowsky method but at times they may show granulations which to some extent may be due to granules deposited from the medium.

Sac Cultures in Rat Serum.—In view of the fact that Prowazek and others are inclined to consider spirochetes as protozoa and cell parasites it was desirable to ascertain whether or not the spirilla could be maintained in active multiplication in a clear serum. Accordingly, the spirilla were inoculated into rat serum, completely freed from corpuscles by centrifugation. Up to the present time we have effected 7 consecutive passages in such serum in the space of 24 days. At each passage a control sac containing defibrinated rat blood was placed in the rat. The serum cultures although totally devoid of corpuscles were in every respect as rich in spirilla as the blood cultures. The conclusion to be deduced from these experiments is that multiplication of spirilla may take place without any intracellular stage. The occasional presence of spirilla in a cell is to be regarded as an accident rather than as an expression of an unrecognized cycle.

A New Flagella Stain for Ps. radicola: F. C. HARRISON.

Take a loop of the mucilaginous or viscid growth from an agar culture of *Ps. radicicola* two days to several months old and spread it on a clean slide, lashing it out in slender tongues, let the film dry in air without killing or fixing, flood the film a moment with a saturated alcoholic solution of gentian violet, wash under the tap, dry between folds of filter paper and examine with the oil immersion lens. The mucilage in which the cells lie will be found deeply and evenly stained, and the bacteria scarcely stained at all, so that the preparation presents the appearance of a photographic negative. The unequal density of the protoplasm of the cells is clearly seen, as indeed it is in the living cells when examined from a hanging drop. (See photograph.)

The single polar flagellum is also clearly demonstrated by this stain since it, like the protoplasm of the cells, refuses the stain, and so it appears as a clear or uncolored streak in the surrounding deeply stained mucilage. The flagella are best stained at the margins of the films and in thin places. In parts of the film where the culture is thickly spread, the mucilage is intensely

stained and the flagella, being slender and enveloped deeply in the mucilage are not distinguished. In these parts, however, the cells are beautifully contrasted with the dark background and their internal structure is clearly shown. Saturated alcoholic solutions of methyl blue, night blue or fuchsin may be used instead of saturated alcoholic gentian violet. A film prepared and stained as above, then flooded an instant with Lugol's solution, is still more intensely and darkly stained. There is no added value to be gained by using any two or even three of these substances together.

Commercial Cultures of Pseudomonas radicicola: H. A. Harding and M. J. Prucha.

During the past two years cultures of *P. radicicola* dried on cotton have been offered commercially to the agricultural public.

At the Ann Arbor meeting we reported the results of an examination of eighteen such cultures, all of which were found to be worthless for practical purposes. A portion of these examinations were done in cooperation with Professor F. D. Chester, of Delaware, Dr. E. M. Houghton and Dr. C. E. Marshall, of Michigan, and Dr. J. G. Lipman, of New Jersey.

Results from tests of similar inoculated cotton cultures have now been given out from sixteen agricultural experiment stations and in only one case have they obtained satisfactory results from such commercial cultures.

Further discussion of these cultures would be needless but for the fact that one of the commercial companies is now putting its product upon the market in metal containers, claiming thereby to obviate the objections which had been raised against the inoculated cotton cultures packed in parchment paper and tin foil as was the case last season.

We have this season examined fourteen commercial cultures of *P. radicicola* which were in such metal containers and find them as worthless as those examined last season.

Bacteria of the Dairy Wells near Washington, D. C.: Karl F. Kellerman and T. D. Beckwith, Bureau of Plant Industry, Department of Agriculture, Washington, D. C.

Sixty wells and springs from as many different dairy farms have been examined and these are believed to fairly represent the conditions obtaining in the 800-odd other dairy water supplies. Of the 60 wells examined, 22 contained below 500 bacteria to the cubic centimeter, and of this number but six showed the presence of *Bacillus coli* in samples of one cubic centimeter. Nine showed the presence of between 500 and 1,000 bacteria to the cubic centimeter, and of these three showed the presence of *B. coli.* Nineteen showed the presence of 1,000 to 5,000 bacteria to the cubic centimeter, and of these ten showed the presence of *B. coli.* Four showed the presence of 5,000 to 10,000 bacteria to the cubic centimeter, and of these three showed the presence of *B. coli.* Six showed the presence of 10,000 to 30,000 bacteria to the cubic centimeter, and of these five showed the presence of *B. coli.*

Some Relations of Bacteria in Milk: C. E. Marshall and Bell Farrand, Michigan Agricultural College.

Influence of Temperature on the Functional Activity of Lactic Bacteria: C. E. Marshall and Louise Rademacher, Michigan Agricultural College.

Lactic Acid Bacteria in Milk: D. H. Bergey, M.D.

Conn and other authorities report the occurrence of two principal types of lactic bacteria in milk. These two types of organisms correspond to *Bacterium lactis acidi* of Leichmann and *Bacterium acidi*

lactici of Hueppe. Kruse regards *Bacterium acidi lactici* as identical with *Bacterium aerogenes*, and Heinemann is of the same opinion. Heinemann believes that *Bacterium lactis acidi* is a myth and that *Streptococcus lacticus* is the principal lactic organism in milk.

A number of samples of 'certified' and 'market' milk were analyzed in order to ascertain the nature of the lactic acid bacteria to be encountered in such milk.

The organisms encountered in certified milk were principally staphylococci and streptococci, the former type being by far the most numerous. Neither of the other types of lactic acid bacteria were found in certified milk, except in isolated instances.

The organisms encountered in market milk were principally rod-shaped organisms, and some of these were of the type of *Bacterium acidi lactici* of Hueppe.

The exact nature of all of the lactic acid bacteria encountered in market milk is still undetermined. Their sugar-splitting powers are believed to assist in a more definite classification.

Commercial Bacterial Inspection of Milk and Its Results: S. C. PRESCOTT.

The problem of providing sweet, clean, and wholesome milk to the inhabitants of large cities is one which demands attention both from the sanitary and the commercial standpoints.

Two distinct lines of action have been suggested: first, to limit the production of milk to individuals who will comply with a strict set of laws or ordinances; and second, to treat milk in some way so as to destroy the bacteria.

After more than eighteen months constant supervision of about two hundred farms I should like to present a record of some of the results of bacterial examination and farm inspection.

It must be accepted, I think, that for a long time to come the major portion of the milk consumed in a large city will be the product of the ordinary farm, handled by ordinary men, and shipped by the ordinary methods of transportation. Too sweeping demands for immediate reforms, or laws which can not at present be obeyed, will serve only to precipitate a milk famine.

In this work I have attempted first of all to visit every farm, and to give the farmer whatever help was possible by suggesting improvements in his methods or appliances, and by explaining the reasons for our inspections. Especial attention was given to the effect of cleanliness and cold. A small pamphlet on clean milk, written in very homely language, has been sent to each producer.

I have graded the milk from these farms for purposes of convenience into six groups, according to the number of bacteria, as follows:

Grade A, Below 10,000 bacteria per cubic centimeter.

Grade B, 10,000 to 50,000 bacteria per cubic centimeter.

Grade C, 50,000 to 100,000 bacteria per cubic centimeter.

Grade D, 100,000 to 250,000 bacteria per cubic centimeter.

Grade E, 250,000 to 500,000 bacteria per cubic centimeter.

Grade F, Above 500,000 bacteria per cubic centimeter.

The percentage of samples exceeding 500,000 fell from 30.5 in June, 1905, when inspection began, to 2.3 in May, 1906; but with the advent of hot weather and the scarcity of ice, rose, as would be expected, to a maximum of 12.1 per cent. in July, 1906, but dropped again as cooler weather approached.

On the other end of the scale, the percentage of samples containing below 10,000 gained from 2.0 in June, 1905, to 37.3 per

cent. in February, 1906, and at no time since has it fallen below 22.2 per cent.

The percentage of samples containing fewer than 50,000 has never fallen below 50 since September, 1905, three months after inspection work began.

Experiments on the Germicidal Action of Fresh Cow's Milk: P. G. Heinemann.

The question whether fresh cow's milk contains bactericidal substances or not has been answered in the affirmative by Fokker, Ehrlich and Brieger, Park, Kolle and his coworkers, Hunziker, Hippius, Koning, and others. In opposition to this, Moro, Honigmann, Basenau, Stocking and others have denied the existence of such substances.

Experiments by the writer carried on by inoculating raw milk and milk heated to 56° C. and to the boiling point indicate that fresh cow's milk contains germicidal substances, although to a smaller degree than blood serum. Milk was obtained directly after milking and divided into three portions, one of which was heated either to boiling or kept at 56° C. for 30 minutes. The second part was left without further treatment, and then both heated and raw milk were inoculated with suspensions of bacterial cultures. The third part was kept as control. Plates were prepared from suitable dilutions and the colonies counted after two days incubation at 37° C. The three lots of milk were kept at room temperature and plating repeated at regular intervals. The results lead to the following conclusions:

Conclusions.—1. Raw milk contains substances which are germicidal to a pronounced degree for some species of bacteria. (*B. coli, B. dysenteriæ* (Flexner), *B. fluorescens, non-liquefaciens.*)

2. Raw milk contains substances which have slight germicidal action on some species of bacteria. (*B. violaceus, B. cholerœ suis, B. prodigiosus* (laboratory culture), and some saprophytes isolated from milk.)

3. The germicidal substances in milk do not act strongly on *B. fluorescens liquefaciens, B. typhosus,* some varieties of *B. prodigiosus* and *B. proteus,* but the multiplication of these organisms is restrained for a limited period.

4. The germicidal action of cow's milk persists for more than 5 hours and less than 7 hours at room temperature.

5. The germicidal action of cow's milk is destroyed by keeping milk at 56° C. for 30 minutes or by heating to the boiling point.

6. The germicidal substances in cow's milk are less powerful than those of blood serum, but are inactivated under similar conditions. The relative concentration of these substances varies in milk from different animals.

S. C. Prescott,
Secretary

SCIENTIFIC BOOKS

Experimentelle Beiträge zur Morphologie. Hefte I. and II. Herausgegeben von Herman Braus (Heidelberg). Leipzig, W. Engelmann. 1906.

The study of experimental morphology, which in recent years has attracted so large a body of enthusiastic students, has been taken up very largely from the dynamic or physiological point of view. This is indicated by the title of the journal most specifically devoted to this line of work—W. Roux's 'Entwicklungsmechanik der Organismen.' Yet, although some physiologists, like Pflüger and Loeb, have done much to stimulate interest in this direction, it is chiefly to professional zoologists and anatomists that the subject has appealed, while the immediate predecessors and many of the contemporaries of these same zoologists and anatomists have been interested rather in phylogenetic and historical than in dynamic biological questions.

The purpose of these Beiträge is to emphasize the value of experimental and accidental